FROZEN BURGERS CONTAINING OREGANO AS NATURAL ANTIOXIDANT: EVALUATION OF REDUCING POWER AND INHIBITORY EFFECT ON PROTEIN OXIDATION

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Abstract – The aim of this study was to assess the influence of oregano natural extract in three concentrations on oxidative parameters of lamb burgers stored for 120 days at -18±1ºC. Five batches were prepared: without antioxidants (control), with sodium erythorbate and with three different volumes of oregano extract as natural antioxidant, according different colorimetric methods: Folin-Ciocalteau (ORE-1), DPPH· (ORE-2) and FRAP (ORE-3). The burgers were submitted to measurements of protein oxidation (carbonyls content assay), reducing power and pH. Regarding carbonyls contents, results did not show differences (P>0.05) during storage time for burgers containing intermediary (ORE-2) and higher (ORE-3) volumes of extract, which presented, at 120 days, lower formation of carbonyls than the samples control and containing sodium erythorbate (P<0.01). With respect to reducing power, the burgers with oregano extract presented higher (P<0.01) values at the end of storage period, and in relation to pH, higher acidity (P<0.05). From the obtained results, one can conclude that the addition of oregano in the higher evaluated volumes, based on DPPH· and FRAP methods, is a viable solution to replace synthetic antioxidant sodium erythorbate, allowing the maintenance of the oxidative stability along storage time and more natural appeal to the meat product.

Key Words – antioxidant capacity, carbonyl, sodium erythorbate.

I. INTRODUCTION

The lipid and protein oxidation processes represent the major causes of quality loss in muscle tissues, mostly due to high concentration of unsaturated lipids, promoting in general, discoloration, off-flavor formation and lower shelf life [1, 2]. More specifically, regarding proteins stability, oxidative reactions are responsible for reducing their solubility and functionality, also causing texture and nutritional changes involving the formation of carbonyl compounds [3, 4].

Currently, to reduce the oxidative process and its consequences, the antioxidants obtained from plants, considered sources of bioactive compounds, are being used in meat and derivatives with a great effectiveness, acting as free radical inhibitors and chelating metal ions [5, 6].

Compared to the synthetic ones, natural antioxidants are related to the healthiness features and enhanced safety. Phenolic compounds are the major components that exert positive effects against oxidative reactions due to their free radical scavenging capacity and reducing power [7].

Thus, the objective of this study was to evaluate the effects of different concentrations of oregano extract added as natural antioxidant in different concentrations on protein oxidation, reducing power and pH values of lamb burgers during 120 days of frozen storage.

II. MATERIALS AND METHODS

A. Preparation of natural extract

The extract of *Origanum vulgare* was obtained in triplicate using acetone, ultrapure water and acetic acid glacial (70/28/2%, respectively) at a

final ratio of the 1:50 (g/mL). After grinding, agitation, centrifugation, filtration, concentration and lyophilization, was performed the ressuspension of the samples in ratio of ¼ (v/v) [8].

B. Evaluation of antioxidant capacity and determination of natural extract volumes

To determine the antioxidant capacity of the extract, methodologies of Folin-Ciocalteau, DPPH$^{\cdot}$ (2,2-diphenyl-1 picrylhydrazyl) and FRAP (Ferric ion Reducing Antioxidant Power) were performed based on Wootton-Beard *et al.* [9]. From these results, the volumes of oregano extract to addition in the meat product were calculated in respect to the equivalence to 500 ppm of sodium erythorbate.

C. Processing of the burgers

The burgers were prepared using lamb meat (84%), and fat trimmings (14%), salt (2%) and antioxidants (natural or synthetic). The meat and fat were thawed at 4 ºC for 12 hours and minced separately using disc of 4 mm. Five batches were manufactured in three repetitions: control without antioxidants (CO), with sodium erythorbate (ER) and with three quantities of natural antioxidant (ORE-1, ORE-2 and ORE-3, relative to Folin, DPPH$^{\cdot}$ and FRAP results of antioxidant capacity, respectively).

The burgers (95-100 g) were formed using a manual molder of 112 mm diameter x 2 cm height and individually separated with polyethylene films. The meat products were frozen in ultra freezer, packed in air-permeable polypropylene bags, and stored at -18 ºC. A total of 30 burgers were analyzed (5 batches x 3 repetitions of manufacture x 2 samples of each batch) in each sampling point.

D. Reducing power of burgers

According to Huang *et al.* [10], with modifications, the samples (0.03 g) were mixed with 2.5 mL of sodium phosphate buffer and 2.5 mL of potassium ferric cyanide 1%. The mixture was incubated at 50 °C for 20 min and were added 2.5 mL of trichloroacetic acid 10% and after, the mixture was centrifuged. The resulting supernatant was mixed with equal volumes of water ultrapure and 1/5 volume of ferric chloride 0.1%. The absorbance was measured at 700 nm after 10 min, based on standard curve with butylated hydroxyanisole (BHA) 0.005 mg/mL. The results were expressed as mg equivalent of BHA/g of sample.

E. Carbonyl groups

The protein content total was quantified at 540 nm based standard curve biuret reagent (0 to 4 mg/mL). 100 µL of homogenate were separately treated with 500 µL of 2N HCl and with 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl. Both samples were incubated for 1h at room temperature and stirred regularly. The samples were precipitated with 20% TCA and centrifuged at 11000 × g for 10 min. The pellets were washed with 1 mL of ethanol:ethyl acetate (1:1, v/v), dissolved in 1 mL of 6 M guanidine HCl and centrifuged again. Protein concentration was calculated at 280 nm and the carbonyl content was quantified through derivatization with DNPH at 370 nm, based on bovine serum albumin (BSA), using a molar absorptivity of 22.000 M-1.cm-1. The results were expressed as nmol of carbonyl/mg of protein [11].

F. pH

The pH was measured in each two samples per batch using a pH meter (HANNA, HI 99163) with combined electrode to perforation in triplicate.

G. Statistical analysis

The results were analyzed by analysis of variance (ANOVA) using the IBM SPSS Statistics 17.0 (IBM Corporation, Somers, NY, USA), and in case of differences mean were compared by Duncan test (5%).

III. RESULTS AND DISCUSSION

From the results of antioxidant capacity were determined the volumes of natural extract to be added into burgers: 1.33, 1.78 and 2.40 mL/100 g, to ORE-1, ORE-2 and ORE-3, respectively.

The influence of natural antioxidant on protein oxidation of frozen lamb burgers during 120 days is shown in Fig. 1.

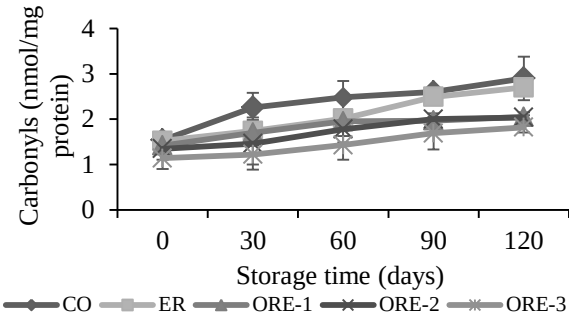

Figure 1. Carbonyl formation during frozen storage.

Carbonyl values presented a gradual trend of increase in all the batches during the storage. However, carbonyl formation in the burgers with natural antioxidant in intermediate and higher concentrations did not differ (P>0.05) from zero time to 120 days presenting higher inhibition of protein oxidation in the meat product.

After 120 days, lower formation of carbonyls in batches containing oregano extract was noted: 2.05±0.10, 2.04±0.05 e 1.82±0.11 nmol/mg of protein in ORE-1, ORE-2 e ORE-3 treatments, respectively, in comparison to CO (2.90±0.48 nmol/mg) and ER (2.70±0.02 nmol/mg) (P<0.01), that did not differ (P>0.05) to each other.

In this present study, the oregano extract was efficient in the inhibition of protein oxidation and this behavior can be probably attributed to the occurrence of covalent bond with phenolic compounds present in the extract, which promoted a higher antioxidant effect [12].

In addition, the measurements of pH confirmed that burgers containing oregano presented lower values (Table 1), as also observed by Lorenzo *et al.* [13] in restructured meat product with some natural antioxidants. This occurred probably by the presence of organic acids, constituents of the extract [14].

With respect to reducing power, the burgers containing oregano extract presented higher values, favored by the presence of antioxidant compounds. In addition, the chain reaction promoted the reduction of ferric cyanide complex with colour modifications [15, 16].

Table 1 pH values of burgers at -18ºC (average values±deviation standard).

Batches	0 days	120 days	Sign.
CO	5.99±0.05[2]	6.05±0.03[2]	n.s.
ER	5.99±0.04[2]	6.05±0.05[2]	n.s.
ORE-1	5.92±0.04[1]	6.00±0.01[1,2]	n.s.
ORE-2	5.91±0.02[1]	5.98±0.03[1]	n.s.
ORE-3	5.89±0.03[1]	5.96±0.02[1]	n.s.
Sign.	*	*	

[1,2,3]: Mean values in the same column (corresponding to the same days of ripening) not followed by a common number differ significantly (P<0.05)
Significance: n.s.: not significant; * (P<0.05); ** (P<0.01); *** (P<0.001).

Based on the calibration curve of BHA, the reducing power was higher in presence of antioxidants and the absorbance was greater. Thus, significant differences (P<0.05) among the batches were detected, but with the same behavior at day zero and at 120 days (P>0.05) (Fig. 2).

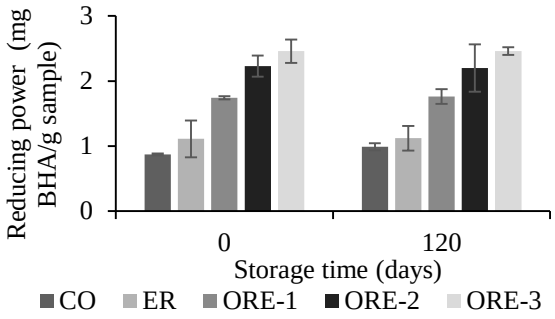

Figure 2. Reducing power of lamb burgers during storage at -18 ºC.

Control and ER presented lower values in comparison to ORE-2 and ORE-3 batches, which did not differ to each other, with average values of 2.23±0.16 e 2.20±0.37 (day zero); e 2.46±0.18 e 2.46±0.06 mg BHA/g of sample (at 120 days), respectively.

During the storage, the oxidative process did not affected in the reducing power of the samples, and in this case, possibly the oxidation may have induced it he formation of reducing compounds,

that contributed to this maintenance [10]. In addition, some proteins and peptides also can present antioxidant action [17], confirmed with results of carbonyls, which were maintained within lower levels until 120 days.

IV. CONCLUSION

According to the results, the maintenance of antioxidant functionality of the oregano extract in the concentrations based on DPPH$^{\cdot}$ and FRAP methods was representative and consequently can represent a promising adjuvant in the maintenance of human health, in addition to the oxidative preservation of lamb burgers during frozen storage.

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